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SHORT COMMUNICATION

Characterization of an Essential HSV-1 Protein Encoded by the UL25 Gene Reported to Be Involved in Virus Penetration and Capsid Assembly

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The 2.3-kb *Bam*HI-U DNA fragment (map units 0.319–0.335) of herpes simplex virus type 1 (HSV-1) genome contains the complete UL25 open reading frame (ORF). It specifies an essential viral protein reported previously to be involved in virus penetration and capsid assembly (C. Addison, F. J. Rixon, J. W. Palfreyman, M. O'Hara, and V. G. Preston, *Virology* 138, 246–259, 1984). To identify the protein encoded by the UL25 gene, the UL25 ORF was cloned in a eukaryotic expression vector (p91023) downstream of the adenovirus major late promoter to generate the expression plasmid p9-UL25. Synthesis of a 60-kDa protein was observed in COS-7 cells transfected with p9-UL25 plasmid DNA, but not in cells transfected with p91023 control plasmid DNA. To identify and characterize the UL25 protein from HSV-1-infected cells, we prepared a rabbit antiserum by using UL25-GST fusion protein expressed in *Escherichia coli* as immunogen. This rabbit antiserum readily immunoprecipitated the 60-kDa UL25 protein from HSV-1-infected cells. In HSV-1-infected cells, UL25 protein was expressed as a late (γ) or a leaky late (γ1) viral protein. The rabbit antiserum raised against HSV-1 UL25 protein immunoprecipitated a UL25-homologue of identical size from HSV-2-infected cells. However, the reactivity of the antiserum with HSV-2 UL25-homologue was weaker than compared to the corresponding HSV-1 protein. Consistent with its classification as a virion component, the UL25 protein was found to be associated with purified HSV-1 virions.

Infection of humans by herpes simplex virus type-1 (HSV-1), a neurotropic alpha-herpesvirus, is initiated by entry of the virus at mucocutaneous surface. The virus entry process is thought to be mediated by the interaction of viral glycoproteins (gC and gB) with the abundant cell surface heparan sulfate proteoglycans which serve as the receptors for the virus (*1, 2*). Following initial attachment to the cell surface entry of HSV into the cells is achieved by fusion of the viral envelope with the cell surface.

The mechanism of virus–cell fusion (virus penetration) and cell–cell fusion (virus spread) with HSV has not been fully characterized. However, at least four viral glycoproteins gB, gC, gD, and gH (*3–11*) are known to mediate virus entry. Glycoprotein C facilitates the initial binding event, whereas gB, gD, and gH are thought to function at a step subsequent to this event. Interestingly, an HSV-1 protein encoded by the essential UL25 gene which lacks sequence motif(s) characteristic of membrane proteins has been reported to play a role in virus penetration. Evidence for this derives from studies with ts1204, a mutant with a defect in the UL25 gene, which at nonpermissive temperature binds strongly to cell surface receptors but is incapable of penetration unless the cells are treated briefly with a fusogen such as polyethylene glycol (PEG) or are shifted to the permissive temperature (*12*). Interestingly, certain ts mutants (e.g., tsB5) and null mutants in gB are similarly impaired, being able to bind to cells but penetrating them only after a brief PEG treatment (*4, 13, 14*).

The UL25 protein is apparently multifunctional since a defect in capsid assembly is also observed if ts1204 is allowed to penetrate cells at permissive temperature before being shifted to nonpermissive temperature. The capsids formed by ts1204 did not contain DNA (*12*), reminiscent of B-type capsid structures. It is well-known that herpesvirus-infected cells contain three type of capsid structures designated A (empty), B (intermediate), and C (full), that can be separated by velocity sedimentation through sucrose gradients (*15–19*). Taken together the studies of ts mutants in the UL25 gene suggest that it is a virion component which functions in virus entry and capsid assembly.

The UL25 gene is located in the *Bam*HI-U fragment contained in the unique long (UL) component of the HSV-1 genome (Fig. 1). DNA sequence analysis has revealed that the UL25 ORF in HSV-1 strain 17syn+ is composed of 580 amino acids, with a predicted molecular weight of 62.6 kDa (*20*). The UL25 gene is conserved among

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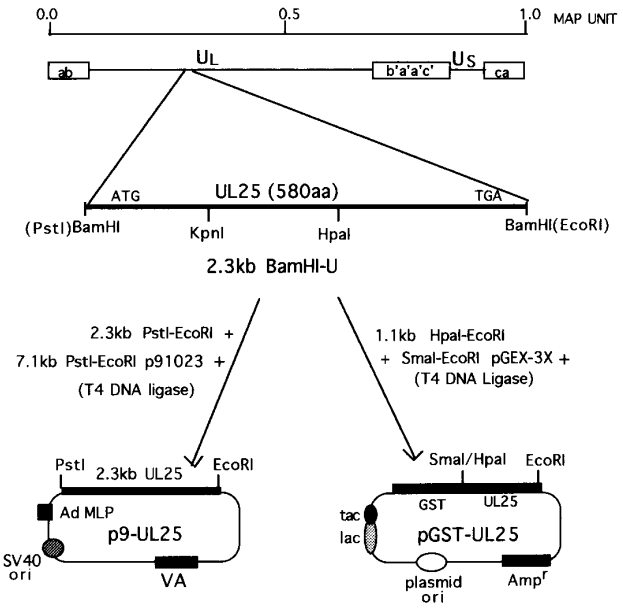


FIG. 1. (Top) Genome location and restriction enzyme (*Bam*HI, *Hpa*I, and *Kpn*I) map of HSV-1 *Bam*HI-U DNA fragment (map units 0.319–0.335); the ATG and TGA codons for UL25 ORF are indicated. (Bottom) Schematic representation of the construction of the eukaryotic expression plasmid p9-UL25 and the bacterial expression plasmid pGST-UL25. Prior to ligation in both construct (p9UL25 and pGST-UL25) the 3′ and/5′ ends were modified to acquire the appropriate restriction sites. The restriction sites in parentheses represent those derived from pUC19 polylinker region.

other alpha-herpesviruses including varicella zoster virus (VZV; gene 34) and equine herpesvirus-1 (EHV-1; gene 36) (*21, 22*). Holland *et al.* (*23*) have identified five transcripts (5.6, 5.2, 4.4, 2.4, and 1.6 kb) originating from the HSV-1 *Bam*HI-U DNA fragment. Of these at least two (5.6 and 5.2 kb) could potentially encode the UL25 protein. However, nothing is known about the protein product specified by the UL25 ORF (*1*). To characterize the UL25 protein, and to study its role in HSV life cycle, we prepared a rabbit polyclonal antiserum to UL25, using a UL25–GST fusion protein expressed in *Escherichia coli* as immunogen.

In this communication, we report the identification and characterization of the UL25 protein of HSV-1. To the best of our knowledge, the present study represents the first report on the expression, identification, and characterization of this essential HSV protein.

To identify and characterize the UL25 protein, we needed specific antibodies. Smith and Johnson (*24*) have developed plasmid vectors that direct the expression of foreign proteins in *E. coli* as chimeric proteins by fusion at the COOH-terminus of sj26, the 26-kDa glutathione *S*-transferase (GST; EC 2.5.1.18) derived from parasite helminth *Schistosoma japonicum.* A UL25–GST fusion protein was expressed using the pGEX-3X vector system (Pharmacia, Piscataway, NJ). Plasmid pGST–UL25 was constructed by ligating a 1.1-kb *Hpa*I–*Bam*HI fragment encoding COOH terminal domain of UL25 protein at the *Sma*I–*Eco*RI site of pGEX-3X. This ligation results in an inframe fusion between GST and 239 amino acid COOH-terminus of HSV–1UL25 protein. A schematic representation of the construction of pGST–UL25 plasmid is given in Fig. 1, together with the map location of UL25 gene in the HSV-1 genome. The DNA sequence of the junction region of GST with UL25 in plasmid pGST–UL25 was determined to confirm inframe ligation (not shown). Upon induction with isopropyl β-D-thiogalacto-pyranoside (IPTG) the plasmid pGST–UL25 directed the synthesis of an approximately 50-kDa UL25–GST fusion protein in *E. coli* which is in agreement with the size predicted for the UL25–GST fusion protein (not shown). The glutathione–Sepharose affinity purified UL25–GST fusion protein was used to immunize rabbits for the production of polyclonal antiserum to HSV-1 UL25 protein. Briefly, a rabbit (R8-3) was immunized with 200–500 μg of purified UL25–GST fusion protein in 0.5 ml of sterile saline mixed with 0.5 ml of Freund's complete adjuvant (DIFCO). Inoculations were 10 subcutaneous injections (0.1 ml per site) on the shaven back. Immunizations were carried out on Days 1, 14, 28; blood collection (from ear vessel) was on Day 21, and thereafter, every other week. Preimmunization serum and antisera were stored at −20°. For radioimmunoprecipitation using HSV-infected CV-1 cells the antisera and preimmune sera were pretreated with acetone powder prepared from CV-1 cells to remove nonspecific reactivity.

To characterize the polypeptide encoded by the UL25 ORF we constructed an expression plasmid p9-UL25 as described in Fig. 1. It contains the 2.3-kb *Bam*HI-U DNA fragment (map units 0.319–0.335) representing the complete UL25 ORF oriented to facilitate expression under the control of adenovirus major late promoter. The 2.3-kb *Bam*HI-U DNA was subcloned from plasmid pSG-18 (*23, 25*) into the eukaryotic expression vector p91023 (*26, 27*). COS-7 cells transfected with p9-UL25 or a control plasmid p91023 lacking an insert were labeled with Tran[^{35}S]-label and the UL25 protein was immunoprecipitated using a rabbit polyclonal antiserum to HSV-1 UL25 protein. The UL25 antiserum specifically immunoprecipitated a 60-kDa protein from p9-UL25-transfected COS cells (Fig. 2A, lane b). This protein band was absent from cell extracts of COS cells transfected with p91023 and immunoprecipitated with UL25 antiserum (Fig. 2A, lane d) or from cell extracts of COS cells transfected with p9-UL25 or p91023 and immunoprecipitated with normal rabbit serum (Fig. 2A, lanes a and c). The UL25 protein expressed in COS-7 cells comigrated on SDS–PAGE with UL25 protein synthesized in HSV-1-infected CV-1 cells (Fig. 2A, lane e), indicating that the two proteins (UL25 expressed in COS-7 cells and UL25 from HSV-1-infected cells) could not be differentiated on the basis of size or electrophoretic mobility. The apparent 60-kDa size of the protein is in good agreement with the pre-

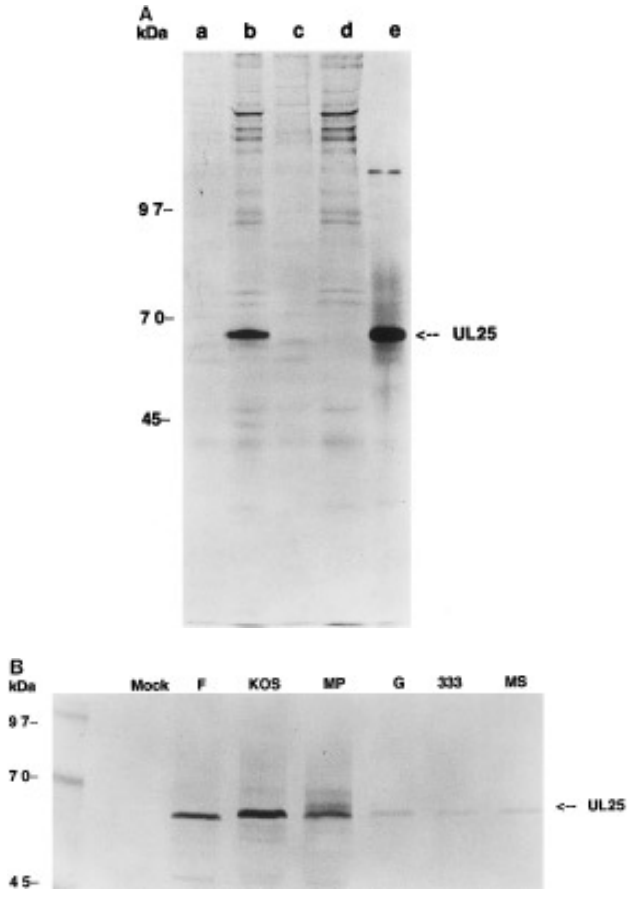


FIG. 2. (A) Expression of HSV-1 UL25 protein in COS-7 cells. COS-7 cells were transfected with p9-UL25 (lanes a and b) or p91023 (lanes c and d) and ^{35}S-labeled proteins were immunoprecipitated using normal rabbit serum (lanes a and c) or rabbit antiserum to HSV-1 UL25 protein (lanes b and d), respectively. Lane e represents UL25 protein immunoprecipitated (using UL25 antiserum) from HSV-1 (strain KOS)-infected CV-1 cells included for the sake of comparison. An autoradiogram of SDS–PAGE gel is shown. The kilodalton values to the left represent protein size markers. (B) Identification of UL25 protein from HSV-1- and HSV-2-infected cells. CV-1 cells were infected with HSV-1 (strains F, KOS or MP) or HSV-2 (strains G, 333, or MS) at a m.o.i. of 5 PFU per cell. At 5 hr after infection cells were labeled with 100 μCi/ml of Tran[^{35}S]-label for 2 hr. HSV UL25 protein was immunoprecipitated using rabbit R8-3 UL25-antiserum. An autoradiogram of electrophoretically separated proteins is shown. The HSV strain used is noted against each lane. Lane mock represents mock-infected cells. Position of UL25 protein is indicated by an arrow mark. The kilodalton values to the left represent protein size markers.

dicted size (62.6-kDa) for the UL25 polypeptide consisting of 580 amino acid (*20*).

Our next objective was to identify the UL25 protein from HSV-infected cells. For these experiments, CV-1 cells were infected with HSV-1 (strain F, KOS, or MP) or HSV-2 (G, 333, or MS) and at 5 hr after infection the cells were labeled with 100 μCi/ml of Tran[^{35}S]-label for 2 hr. The HSV-1 UL25 or its homologue in HSV-2-infected cells was immunoprecipitated using the rabbit antiserum to UL25 and the proteins were analyzed by denaturing polyacrylamide gel electrophoresis (SDS–PAGE). The UL25 antiserum specifically immunoprecipitated a 60-kDa protein from HSV-1 (strains F, KOS, and MP)-infected cells as shown in Fig. 2B. An analogous 60-kDa protein was also immunoprecipitated from HSV-2 (strains G, 333, and MS)-infected cells, but the intensity of the band was much weaker (Fig. 2B). These results show that the 580 amino acid open reading frame in the unique long (UL) component of HSV-1 genome designated UL25 is expressed as a 60-kDa protein in CV-1 cells infected with three different HSV-1 and HSV-2 strains, respectively. The apparent reduced expression of the HSV-2 UL25 homologue in infected cells was not the consequence of reduced HSV-2 replication because comparable amounts of the 140-kDa ribonucleotide reductase large subunit (RR1 protein) were immunoprecipitated from the same extracts of HSV-1- and HSV-2-infected cells with the 51S mAb (not shown; *28*). Thus, the UL25 homologue expressed by the HSV-2 strains reacted weakly with HSV-1-specific antibody (Fig. 2B), indicating that the HSV-1 and HSV-2 UL25 proteins have diverged, at least in the 239-aa COOH-terminal domain that was used to elicit the polyclonal antiserum. It is interesting to note that the UL25 mutant, ts1204, when bound to the cells at the nonpermissive temperature blocks superinfection by HSV-1 but not by HSV-2 (*12*). This has been suggested to be the consequence of the use of different cell surface receptors by HSV-1 and HSV-2. An alternative explanation is that the mutant UL25 protein transdominantly interferes with the function of wild-type HSV-1 UL25 protein. We speculate that HSV-2 penetration would be unaffected because sequence divergence of the HSV-2 UL25 protein would preclude it from being susceptible to the transdominant effects of the mutant HSV-1 UL25 protein.

To establish the temporal class of the UL25 protein, the kinetics of its synthesis were examined by pulse chase labeling experiments. CV-1 cells were mock-infected or infected with HSV-1 and at various times after infection cells were pulse-labeled for 1 hr with 100 μCi/ml of Tran[^{35}S]-label. Cells were harvested following a 30-min chase with a 50-fold excess of unlabeled L-methionine and L-cysteine, during which time any incompletely synthesized or precursor forms of the protein were converted into the mature form. The UL25 proteins were immunoprecipitated from cell extracts using rabbit UL25 antiserum. As controls the immediate-early (IE or α) ICP4 and ICP6 and early (E or β) gB proteins were immunoprecipitated from the same cell extracts using 58S, 51S, and 3S mAbs (*29–31*), respectively, to examine the kinetics of synthesis of these proteins in parallel with UL25. The immunoprecipitated proteins separated on 10% SDS–PAGE were visualized by fluorography. The results are shown in Fig. 3. Synthesis of 60-kDa UL25 protein begins as early as 2 hr after infection (Fig. 3). Maximal (peak level) synthesis of UL25 was found to occur at 6–9 hr postinfection, declining to minimal levels at 16 hr postinfection. Maximal (peak level) synthesis of HSV-1 ICP4

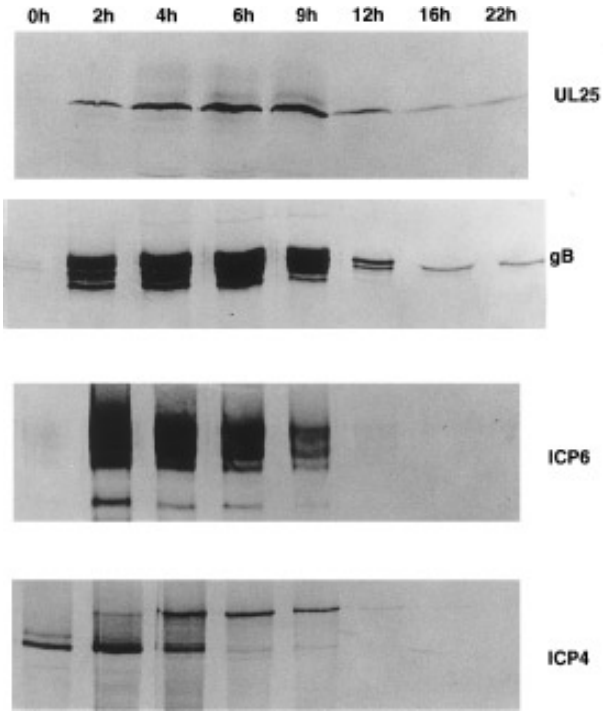


FIG. 3. Kinetics of UL25 protein synthesis in HSV-1-infected cells. CV-1 cells were mock-infected or infected with HSV-1 (strain F) at a m.o.i. of 5 PFU per cell. At various times after infection cells were labeled with 100 μCi/ml of Tran[^{35}S]-label for 1 hr in a L-methionine- and L-cysteine-deficient medium and chased with a medium containing 50-fold excess of L-methionine and L-cysteine for 30 min. UL25 protein was immunoprecipitated from cell extracts using a rabbit UL25 antiserum (R8-3). Glycoprotein gB, ICP6, and ICP4 proteins were immunoprecipitated using mAbs 3S, 51S, and 58S, respectively. Immunoprecipitated proteins were analyzed on 10% SDS–PAGE followed by fluorography. Autoradiogram of electrophoretically separated proteins immunoprecipitated from HSV-1-infected CV-1 cells is shown. The time points at which radiolabeling was initiated is noted against each lane. 0-hr time point actually represents proteins immunoprecipitated from infected cells (extracts) labeled with Tran[^{35}S]-label for 1 hr and then chased for 30 min with unlabeled L-methionine and L-cysteine following a 1-hr adsorption step. 2-hr time point includes a 1-hr adsorption step, 2-hr infection step, and subsequent labeling and chase as in 0 hr. The specific HSV-1 protein analyzed is noted on the right hand side of each panel.

and ICP6 (RR1) were found to occur at 2 hr postinfection, then declining to undetectable levels at 12 hr postinfection. Although not visible in this exposure, peak-level synthesis of gB was found to occur at 6 hr postinfection and then declining to minimal levels at 16 hr postinfection in a manner similar to that observed for the UL25 protein. Since glycoprotein B is known to be an early (β) protein of HSV-1, it was considered likely that the UL25 protein belonged to the early (β) class of HSV-1 proteins. No protein product characteristic of UL25 was observed in mock-infected CV-1 cells as compared to HSV-1-infected cells analyzed under identical conditions (not shown). The other proteins ICP4 and ICP6 analyzed under identical conditions showed a pattern of biosynthesis characteristic of IE (α) proteins. The finding that ICP6 (RR1) biosynthesis pattern is identical to that of ICP4 is consistent with the presence of the sequence motif TAATGARAT characteristic of IE genes reported by Wymer *et al.* (*32*) for the HSV-2 RR1 gene and Desai *et al.* (*33*) for the HSV-1 RR1 gene, respectively. The two forms of ICP4 observed at IE (0–4 hr) and early (4–9 hr) times after infection are probably related to the posttranslational modification of this protein (*34*). We should note that a control for a late (γ) protein of HSV-1 such as gC was not included in this experiment.

To distinguish between the possibility that the UL25 protein is an early (β) or a late (γ) HSV-1 protein, we next determined whether the viral DNA replication was required or not for UL25 synthesis. For these experiments, CV-1 cells were infected with HSV-1 and maintained in the presence or absence of 300 μg of phosphonoacetic acid (PAA; Aldrich) per milliliter added at the onset of infection (*35*). Infected cells were labeled with 100 μCi/ml of Tran[^{35}S]-label between 3 and 8 hr after infection. The radiolabeled UL25 protein, ICP4 (as an example of IE or α protein), gB (as an example of E or β protein), and gC (as an example of L or γ protein) were immunoprecipitated from the same cell extracts using antibodies specific to each of these proteins. UL25 protein was immunoprecipitated by using rabbit UL25 antiserum. ICP4 and glycoproteins gB and gC were immunoprecipitated using 58S, 3S, and 5S mAbs, respectively (*29*). Immunoprecipitated proteins were separated by SDS–PAGE and located by fluorography. The results are shown in Fig. 4. Expression of glycoprotein gC, a late or γ gene product was diminished to 30% of the control level in the presence of PAA (Fig. 4; lanes gC, $-$ and $+$ PAA). In contrast, the expression of immediate-early (α protein) ICP4 went up by about 15% in the presence of PAA (Fig. 4; lane ICP4, $-$ or $+$ PAA). Accumulation of glycoprotein gB, an early or β gene product appears to remain unchanged in the presence of PAA. There was a decrease in the accumulation of UL25 protein in the presence of PAA in a manner similar to that observed with HSV-1 gC (Fig. 4; lanes gC and UL25, $-$ or $+$ PAA). However, the extent of reduction in the accumulation of UL25 was significantly less than compared to HSV-1gC, a true late (γ2) viral protein (Fig. 4). Based on these results it is appropriate to classify UL25 as a late (γ) or leaky late (γ1) HSV-1 protein.

McGeoch and colleagues (*20, 36*) have classified the product of HSV-1 UL25 gene as a virion protein. To demonstrate that the UL25 protein is a virion component, extra-cellular HSV-1 virions labeled with Tran[^{35}S]-label were purified by velocity sedimentation in sucrose gradient essentially according to published methods (*37–39*). Briefly, CV-1 cells (grown on two T175 flasks) were infected with HSV-1 (F) at a m.o.i. (multiplicity of infection) of 5 PFU (plaque forming units) per cell. Infected cells were labeled from 6 to 36 h after infection with 50 μCi/ml of Tran[^{35}S]-label. Extracellular virus concentrated by

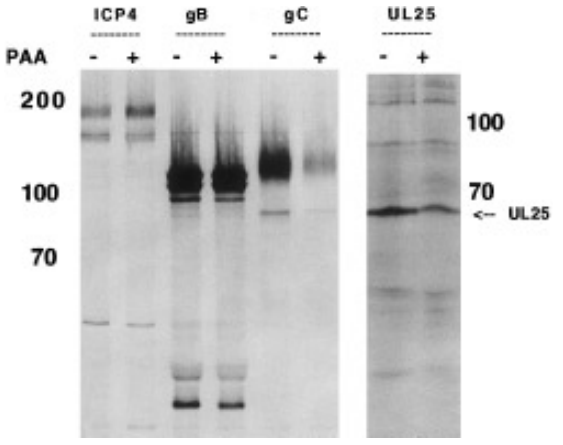
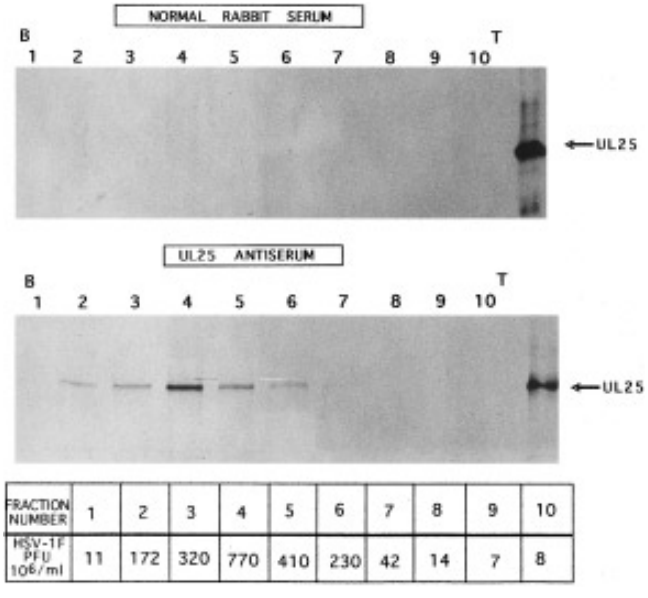


FIG. 4. Expression of UL25 protein in the presence of phosphonoacetic acid (PAA), a DNA synthesis inhibitor. CV-1 cells were infected with HSV-1 at a m.o.i. of 10 PFU per cell in the presence of 300 μg/ml or no PAA added after 1 hr adsorption step. Cells were labeled with Tran[^{35}S]-label between 3 and 8 hr postinfection. UL25 protein was immunoprecipitated from cell extracts using a rabbit UL25 antiserum. ICP4, and glycoproteins gB and gC were immunoprecipitated using monoclonal antibodies (mAbs) 58S, 3S, and 5S, respectively. Immunoprecipitated proteins were analyzed on 10% SDS–PAGE followed by fluorography. Depicted in the figure is an autoradiogram of electrophoretically separated proteins immunoprecipitated from HSV-1-infected CV-1 cells. The data on radioimmunoprecipitation of UL25 using rabbit antiserum, and that on ICP4, gB, or gC radioimmunoprecipitated using mAbs was derived from separate autoradiograms. Percentage inhibition (see text) was calculated based on cpm immunoprecipitated using the corresponding mAbs in the presence or absence of PAA. Numbers represent protein size markers in kDa.

centrifugation was layered on a 10-ml 20 to 60% (w/v) continuous sucrose gradient. Centrifugation was at 20,000 rpm for 20 hr at 4° in a SW41 rotor. Ten fractions (about 1 ml each) were collected from the bottom of the gradient, and the UL25 protein was immunoprecipitated from (0.5-ml aliquot) each fraction using rabbit polyclonal UL25 antiserum. Another aliquot (0.5 ml) of each fraction was immunoprecipitated using normal rabbit antiserum. The HSV-1 virus titers (PFU/ml) were determined for each fraction (using desired dilution from a 10-μl aliquot) by plaque assay on monolayers of CV-1 cells. Shown in Fig. 5 are the results from this sedimentation analysis, together with HSV-1 titers (PFU per ml) determined for each of the fractions. It is clear that the 60-kDa UL25 protein (peak fraction 4; Fig. 5, autoradiogram) and the peak of infectious virus particles (fraction 4, see table in Fig. 5) cosediment on a 20–60% sucrose gradient, indicating that the UL25 protein is associated with HSV-1 virions. Similar results (cosedimentation of UL25 protein and virus infectivity) were obtained when radiolabeled extracellular virions were purified by centrifugation through 5 to 15% Ficoll gradient essentially as described by Szilagyi and Cunningham (*40;* not shown). The finding that UL25 protein is associated with HSV-1 virions is consistent with the suggestion of Addison and colleagues (*12*) and the classification of McGeoch *et al.* (*20, 36*). Sedimentation analysis in sucrose gradients has been used in previous studies to show that HSV-1 ICP4 (*37*) and ICP0 (*38*) and HSV-2 ribonucleotide reductase (*39*) proteins are associated with purified infectious virions. Sequence analysis of the UL25 ORF (*1, 20*) failed to identify motifs that typify membrane proteins and thus the UL25 protein may be a component of the tegument. It is now known that sucrose gradients do not adequately separate virions from L-particles (tegument-envelope structures) and therefore a remote possibility does exist that the UL25 protein is a component of L-particles and not the virions. The existence of UL25 ts mutants makes for a strong case that the UL25 protein is a component of the virions.

In conclusion, we have expressed a GST–UL25 fusion protein and raised antibodies in rabbits to the fusion protein; using UL25 antibody, it was shown that UL25 is expressed in the absence of prior viral DNA synthesis. However, its accumulation was inhibited in the presence of the inhibitor, consistent with it being an leaky late (γ1) viral protein. Additionally, the UL25 protein was shown to be a component of the virion, in all probability the tegument. The UL25 antibody also reacted with HSV-2 UL25 homologue, albeit less strongly, reflecting sequence divergence from the corresponding HSV-1 pro-



FRACTION NUMBER	1	2	3	4	5	6	7	8	9	10
HSV-1F PFU 10^6/ml	11	172	320	770	410	230	42	14	7	8

FIG. 5. Cosedimentation of HSV-1 UL25 protein with infectious HSV-1 particles. Extracellular HSV-1 labeled with Tran[^{35}S]-label was applied on a continuous 20 to 60% sucrose gradient. Centrifugation was for 20 hr at 20,000 rpm in a SW41 rotor (4°). Fractions (about 1 ml) were collected from the bottom of the tube and the UL25 was immunoprecipitated from a 0.5-ml aliquot of each fraction using rabbit UL25 antiserum. Control immunoprecipitations were done in parallel using normal rabbit serum. An autoradiogram of electrophoretically separated proteins is shown together with the HSV-1 titers (PFU per ml) determined for each fraction. The fraction number is noted against each lane. B and T refer to the bottom and top of the gradient tube, respectively. The position of UL25 protein is indicated by an arrow mark.

tein as the most likely possibility. In addition, the transient expression of the 60-kDa UL25 protein in COS-7 cells reported here provides the ground work for generating cell lines expressing this essential HSV protein. An in depth analysis of the role of UL25 protein will be possible once the null mutants in UL25 are isolated using cell lines expressing this essential HSV protein.

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REFERENCES

1. Spear, P. G., *In* "Viral Fusion Mechanisms" (J. Bentz, Ed.,), pp. 201–232. CRC Press, Boca Raton, FL, 1993.
2. Herold, B. C., WuDunn, D., Soltys, N., and Spear, P. G., *J. Virol.* 65, 1090–1098 (1991).
3. Cai, W., Person, S., Warner, S. C., Zhou, J., and DeLuca, N. A., *J. Virol.* 61, 714–721 (1987).
4. Cai, W., Gu, B., and Person, S., *J. Virol.* 62, 2596–2604 (1988).
5. Desai, P. J., Schaffer, P. A., and Minson, T., *J. Gen. Virol.* 61, 1147–1156 (1988).
6. Forrester, A., Farrel, H., Wilkinson, G., Kaye, J., Davis-poynter, N., and Minson, T., *J. Virol.* 66, 341–348 (1992).
7. Fuller, A. O., and Spear, P. G., *Proc. Natl. Acad. Sci. USA* 84, 5454–5458 (1987).
8. Fuller, A. O., Santos, R., and Spear, P. G., *J. Virol.* 63, 3435–3443 (1989).
9. Highlander, S. L., Sutherland, S., Gage, P. J., Johnson, D. C., Levine, M., and Glorioso, J. C., *J. Virol.* 61, 3356–3364 (1987).
10. Highlander, S. L., Cai, W., Person, S., Levine, M., and Glorioso, J. C., *J. Virol.* 62, 1881–1888 (1988).
11. Ligas, M., and Johnson, D. C., *J. Virol.* 62, 1486–1494 (1988).
12. Addison, C., Rixon, F., Palfreyman, J., O'Hara, M., and Preston, V. G., *Virology* 138, 246–259 (1984).
13. DeLuca, N. A., Bzik, D., Bond, V. C., Person, S., and Snipes, W., *Virology* 122, 411–423 (1982).
14. Sarmiento, M., Haffey, M., and Spear, P. G., *J. Virol.* 29, 1149–1158 (1979).
15. Gibson, W., and Roizman, B., *J. Virol.* 10, 1044–1052 (1982).
16. Perdue, M. L., Cohen, J. C., Kemp, M. C., Randall, C. C., and O'Callaghan, D. J., *Virology* 59, 201–216 (1974).
17. Perdue, M. L., Cohen, J. C., Randall, C. C., and O'Callaghan, D. J., *Virology* 64, 187–204 (1975).
18. Perdue, M. L., Cohen, J. C., Kemp, M. C., Randall, C. C., and O'Callaghan, D. J., *Virology* 74, 194–208 (1976).
19. Rixon, F. J., *Semin. Virol.* 4, 135–144 (1993).
20. McGeoch, D., Dalrymple, M., Davison, A., Dolan, A., Frame, M. C., McNab, D., Perry, L. J., Scott, J. E., and Taylor, P., *J. Gen. Virol.* 69, 1531–1574 (1988).
21. Davison, A. J., and Scott, J. E., *J. Gen. Virol.* 67, 1759–1816 (1986).
22. Telford, E., Watson, M., McBride, K., and Davison, A., *Virology* 189, 304–316 (1992).
23. Holland, L. E., Sandri-Goldin, R. M., Goldin, A. L., Glorioso, J. C., and Levine, M., *J. Virol.* 49, 947–959 (1984).
24. Smith, B. D., and Johnson, K. S., *Gene* 67, 31–40 (1988).
25. Goldin, A. L., Sandri-Goldin, R. M., Levine, M., and Glorioso, J. C., *J. Virol.* 38, 50–58 (1981).
26. Kaufman, R. J., *Proc. Natl. Acad. Sci. USA* 82, 698–693 (1985).
27. Ali, M. A., Butcher, M., and Ghosh, H. P., *Proc. Natl. Acad. Sci. USA* 84, 5675–5679 (1987).
28. Ali, M. A., McWeeney, D., Milosavljevic, A., Jurka, J., and Jariwalla, R. J., *Proc. Natl. Acad. Sci. USA* 88, 8257–8261 (1991).
29. Showalter, S. D., Zweig, M., and Hampar, B., *Infect. Immunol.* 34, 684–692 (1981).
30. Ali, M. A., *Virology* 178, 588–592 (1990).
31. Ali, M. A., Prakash, S. S., and Jariwalla, R. J., *Virology* 187, 360–367 (1992).
32. Wymer, J. P., Chung, T. D., Chang, Y-N., Hayward, G. S., and Aurelian, L., *J. Virol.* 63, 2773–2784 (1989).
33. Desai, P., Ramakrishnan, R., Lin, Z. W., Osak, B., Levine, M., and Glorioso, J. C., *J. Virol.* 67, 6125–6135 (1993).
34. Roizman, B., and Sears, A. E. *In* "Virology" (B. Fields and D. Knipe, Eds.), 2nd ed., pp. 1795–1841. Raven Press, New York, 1990.
35. Honess, R. W., and Watson, D. H., *J. Virol.* 21, 584–600 (1977).
36. McGeoch, D. J., Barnett, B. C., and MacLean, C. A., *Semin. Virol.* 4, 125–134 (1993).
37. Yao, F., and Courtney, R. J., *J. Virol.* 63, 3338–3344 (1989).
38. Yao, F., and Courtney, R. J., *J. Virol.* 66, 2709–2716 (1992).
39. Ali, M. A., *Virology* 207, 409–416 (1995).
40. Szilagyi, J. F., and Cunningham, C., *J. Gen. Virol.* 62, 661–668 (1991).